CLAIMS

Claim 1. A method for preparing polynucleotide fragments for use in polynucleotide shuffling, comprising exposing at least one homologous heteroduplex polynucleotide to a polynucleotide repair system until said heteroduplex polynucleotide comprises at least one annealed fragment; and denaturing said heteroduplex polynucleotide to obtain said fragment.

Claim 2. The method of claim 1, comprising fragmenting at least one strand of said heteroduplex polynucleotide by further exposing said heteroduplex polynucleotide to a DNase or restriction enzyme.

Claim 3. The method of claim 1 or 2, wherein the steps occur *in vitro.*

Claim 4. The method of claim 2, wherein said fragmenting comprises fragmenting with at least one restriction enzyme which has multiple cutting sites, or with a plurality of different restriction enzymes.

Claim 5. The method of claim 4, wherein the fragments are at least 15 residues in length.

Claim 6. The method of claim 1, wherein said heteroduplex polynucleotide is generated from a native gene by successive directed mutagenesis, by error-prone PCR, by random chemical mutagenesis, by *in vivo* random mutagenesis, or by combining genes from gene families within the same or different species.

Claim 7. The method of claim 1, wherein said fragments are non-identical.

Claim 8. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides and before exposing said heteroduplex polynucleotide to a polynucleotide repair system, promoting formation of said heteroduplex polynucleotide by

increasing the number of a parent polynucleotide in said library relative to other parent polynucleotides in said library.

Claim 9. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides and before exposing said heteroduplex polynucleotide to a polynucleotide repair system, promoting formation of said heteroduplex polynucleotide by denaturing and rehybridizing the parent polynucleotides.

Claim 10. The method of claim 1, wherein said polynucleotide repair system is a mismatch repair complex, a base excision repair complex, a nucleotide excision repair complex, phage T4 endonuclease VII, phage T7 endonuclease I, or a combination thereof.

Claim 11. The method of claim 10, wherein said mismatch repair complex is DAM methylase, MutS, MutL, MutH, exonuclease, DNA helicase II, SSB protein, DNA polymerase III, DNA ligase, or a combination thereof.

Claim 12. The method of claim 10, wherein said base excision repair complex is DNA glycosylase, AP endonuclease, DNA polymerase I, DNA ligase, or a combination thereof.

Claim 13. The method of claim 10, wherein said nucleotide excision repair complex is Uvr-A, Uvr-B, Uvr-C, DNA polymerase I, DNA ligase, or a combination thereof.

Claim 14. The method of claim 1, wherein exposing said heteroduplex polynucleotide to a polynucleotide repair system comprises incubating said parent polynucleotides with phage T4 endonuclease VII, phage T7 endonuclease I, or a conibination thereof.

Claim 15. The method of claim 4, wherein the fragments are about 15 residues in length to about X residues in length, wherein X equals one residue less

than the total number of residues in the longest polynucleotide in the reaction mixture.

Claim 16. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides and before exposing said heteroduplex polynucleotide to a polynucleotide repair system, introducing at least one mismatch per parent polynucleotide.

Claim 17. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides, and at least one strand of the parent polynucleotides is methylated.

Claim 18. The method of claim 1, wherein said heteroduplex polynucleotide comprises dITP or uracil-containing DNA.

Claim 19. The method of claim 1, wherein said heteroduplex polynucleotide comprises heteroduplex between DNA and RNA.

Claim 20. The method of claim 1, wherein said polynucleotide repair system lacks polymerase, ligase or both.

Claim 21. The method of claim 1, wherein said polynucleotide repair system only partially digests and partially cleaves mismatches.

Claim 22. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides, and wherein at least one damaged base is introduced per initial parent polynucleotide.

Claim 23. The method of claim 1, wherein said heteroduplex polynucleotide is obtained from a starting library of parent polynucleotides, and wherein at least one damaged nucleotide is introduced per initial parent polynucleotide.

Claim 24. The method of claim 1, wherein the steps occur *in vivo*.

Claim 25. A polynucleotide shuffling reaction mixture comprising fragments of at least at two homologous heteroduplex polynucleotides, wherein said fragments were created at least in part by a polynucleotide repair system that lacked DNA polymerase.

Claim 26. A polynucleotide shuffling reaction mixture comprising fragments of at least two homologous heteroduplex polynucleotides for which damaged or mismatched residues have been repaired.

Claim 27. A polynucleotide shuffling reaction mixture comprising at least two homologous heteroduplex polynucleotides wherein at least one strand of said heteroduplex polynucleotides comprises fragments created at least in part by a polynucleotide repair system that lacked DNA polymerase.